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Note

Screening of Edible Plants for Reducing Activity by Monitoring Their Effects on the Oxidation of Oxymyoglobin

Shinya ASHIDA[1]*, Ryoichi SATO[1] and Minoru SATO[2]

[1] *Central Research Institute, Maruha Group Inc., 16–2 Wadai, Tsukuba, Ibaraki 300–4295, Japan*

[2] *Laboratory of Marine Biochemistry, Graduate School of Agricultural Science, Tohoku University, 1–1 Tsutsumi-dori Amamiya , Aoba, Sendai, Miyagi 981–8555, Japan*

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The ability to determine the total reductant capacity (*i.e.* the total amount of electron-donating antioxidants) in dietary foodstuffs would be useful, because foods contain a number of different components with reducing activity. We assessed reducing activity in a variety of edible plants, including various fruits, vegetables, roots, and tubers. The reductive effect was assessed by measuring the ability of each sample to inhibit the oxidation of oxymyoglobin (MbO_2) to metmyoglobin (metMb). We found that several types of plant, such as Chinese cabbage, lemon, paprika, and radish, show marked inhibitory effects on MbO_2 oxidation. Using the MbO_2 assay, it was determined that L-ascorbic acid (AsA) was the main reductive substance in these active plants. However, the majority of the plants tested, including herbs that are regarded as being abundant in antioxidants, were found to promote MbO_2 oxidation. The results of the present study may be useful in the identification of beneficial dietary foodstuffs.

Keywords: reducing activity; reductants; oxymyoglobin; L-ascorbic acid; edible plants

Introduction

Reductants in foodstuffs, such as L-ascorbic acid (AsA), which have the ability to donate electrons and eliminate active oxygen species *in vivo,* have recently attracted a great deal of attention. To evaluate reducing activity in a variety of foodstuffs, various assay methods employing reduction of an inorganic substance, such as ferricyanide (Arai, 1998), or that of a synthetic organic substance, such as XTT (Ukeda *et al.,* 1995), have been reported. An automated test of reducing capability, the ferric-reducing ability of plasma (FRAP) assay, which uses a 2,4,6-tri-pyridyl-s-triazine-iron complex, has been developed (Benzie and Strain, 1996). However, it is questionable whether these methods employing inorganic or synthetic organic compounds are directly applicable to the evaluation of the reducing capabilities in complex systems, such as food materials or organisms.

We developed a new spectrophotometric assay system for evaluating reducing capability using a naturally occurring organic substance, native bovine oxymyoglobin (MbO_2) (Ashida *et al.,* 2004). After incubation of a MbO_2 solution at fixed temperature and pH, the autoxidation rate to metmyoglobin (metMb) is measured and the reductive effects of test samples toward the autoxidation of MbO_2 may be estimated based on changes in the rate constants of conversion from MbO_2 to metMb. Based on comparisons of the results obtained for water-soluble low-molecular-weight compounds using the MbO_2 assay with the data obtained by ferricyanide and XTT assays, the MbO_2 assay was shown to be useful due to its superior sensitivity, application flexibility, and ability to evaluate not only reducing capacity but also "pro-oxidant" activity.

In the present study, the MbO_2 assay was used to examine the reductive effects of dietary foodstuffs. As a new approach for the effective evaluation of the total reductant capacity of in a variety of dietary foodstuffs, a screening test for inhibitory activities towards MbO_2 oxidation was performed on water extracts from 55 edible plants. Moreover, four plants—Chinese cabbage, lemon, paprika, and radish— which showed marked reductive abilities during screening, were examined at different concentrations to determine their effects and the source of their reductive activities.

Materials and Methods

Materials Non-frozen fresh beef (*musculus biceps femoris* of Japanese black cattle) for the preparation of MbO_2 and edible plants to be screened for reductive activity were purchased at supermarkets in Tsukuba City. Ascorbate oxidase (AsOD) from cucumber (activity about 200 units/mg) was purchased from Oriental Yeast Co., Ltd (Tokyo, Japan). Ascorbate oxidase was dissolved in 0.4 M phosphate buffer (pH 6.0) at a concentration of 10 mg/L. Before

* To whom correspondence should be addressed.
E-mail: s-ashida@maruha.co.jp

addition of the MbO_2 solution, the sample filtrate solution and phosphate buffer containing AsOD were mixed and pre-incubated at room temperature for 20 minutes. The final concentration of AsOD in the reaction mixture was 2.5 mg/L. All other reagents were purchased from Wako Pure Chemical Industries (Osaka, Japan). Milli-Q water was used in all procedures.

Edible plant sample preparation After removal of the non-edible part, each plant sample was diced and homogenized in a 5-fold excess of water by weight, extracted by incubation for 10 min at room temperature and then filtered through a filter paper. The inhibitory effects of the filtrates on the oxidation of MbO_2 were measured within 30 min after the preparation.

Chinese cabbage, lemon, paprika, and radish, which showed high inhibitory values in screening, were prepared as freeze-dried powders for further detailed examination. Diced plant samples with the non-edible parts removed were homogenized with a food processor and freeze-dried, then powdered with a mill and stored at −20℃. Before measurement of oxidation, each freeze-dried sample was added to a 100-fold excess of iced water by weight, mixed, extracted at 0℃ for 10 min, and filtered through a disposable filter (pore size 0.45 μm). Immediately after preparation, the filtrate, as the original sample solution, was used for measurement of the autoxidation rate of MbO_2 at four different concentrations (1: 10, 1: 30, 1: 100, and 1: 300 dilutions).

Preparation of MbO_2 The preparation of MbO_2 from bovine muscle was performed in a manner similar to that described in our previous report (Ashida *et al.,* 2004), according to a modification of the method of Trout and Gutzke (1996). All subsequent procedures were carried out at low temperatures (0–5℃) to minimize the production of metMb. The central part of a beef block, from which the colored surface layer had been removed, was diced and homogenized in cold buffer solution (10 mM Tris-HCl (pH 8.0) containing 1 mM EDTA). The homogenate was centrifuged at 10,000 g for 10 min. After adjustment of the pH to 8.0, the supernatant was fractionated with ammonium sulfate (65–100% (w/v) saturation). The precipitate containing Mb was suspended in cold 5 mM Tris-HCl buffer (pH 8.5) and then passed through a Sephadex G-100 column (26ϕ×850 mm, Pharmacia Co., Uppsala, Sweden) with 5 mM Tris-HCl buffer (pH 8.5), and fractions were collected. Each fraction was monitored for absorbance at 730, 572, 525, and 280 nm with a Hitachi Model U-3210 spectrophotometer (Hitachi Ltd., Tokyo, Japan). The Mb fractions that had an A_{280}/A_{525} ratio <5.2 were pooled. The Mb solution obtained as described above was kept at 0℃ in the dark and in the presence of oxygen until use. The prepared MbO_2 was stable and showed little conversion to metMb for at least three weeks in storage.

Measurement of the autoxidation rate of MbO_2 Measurements were carried out at 30℃ according to our standard procedure(Ashida *et al.,* 2004). Before measurement, the Mb solution was desalted and concentrated with an ultrafiltration unit (USY-1, Advantec Toyo Ltd., Tokyo, Japan) at an oxygen pressure of 0.2 MPa for about 2 h at 0℃. The concentrated Mb pellet on the USY-1 filter cup was diluted with distilled water, and the concentration of MbO_2 was set to 60 μM. The concentration of MbO_2 was determined using the following formula: [Mb] (μM)=0.132 ×($A_{525}-A_{730}$) (Krzywicki, 1982). The rate of autoxidation of MbO_2 was measured as follows: 3 ml of the reaction mixture containing MbO_2 (30 μM), 0.1 M phosphate buffer (pH 6.0) and a fixed concentration of the sample filtrate solution was incubated. The phosphate buffer was kept at 0℃ and air-equilibrated by bubbling with air just before addition. The absorbances of the reaction mixtures were measured at three wavelengths (730, 572, and 525 nm) over a period of one hour. To eliminate the absorbance of the sample itself, the reaction mixtures without MbO_2 were measured as blanks. To calculate the rate constant for autoxidation of MbO_2 to metMb, 0% and 100% metMb standard solutions were prepared by addition of sodium hydrosulfite or potassium ferricyanide (about 1 mg), respectively. The percentage of MbO_2 in the sample mixture could then be calculated by comparing of the absorbance ratios of the sample solution (($A_{572}-A_{730}$)/($A_{525}-A_{730}$)) with the corresponding values for the 0% and 100% metMb standard solutions (Krzywicki, 1982). The autoxidation process was followed by plotting the logarithm of the percentage of MbO_2 *vs.* time, and the apparent rate constant values, k_{obs}, for autoxidation of MbO_2 to metMb were determined from the slope of each straight line in the first-order plot. For screening, the reaction mixtures were incubated in polyethylene tubes in a water bath at 30℃, and to simplify the measurement, k_{obs} was determined at measurement at only two time points (0 and 60 min). In experiments other than those performed during the screening procedure, the mixtures were incubated in glass cuvettes with 1-cm path width at 30℃ in a spectrophotometer (U-3210, Hitachi) equipped with a temperature-controlled cell holder, and k_{obs} was determined by measurement at 9 time points at intervals of 5 min for 20–60 minutes, as described in our previous report (Ashida *et al.,* 2004).

The inhibitory rate (IR) of each sample can be expressed by the following formula: {(k_{obs} of control)−(k_{obs} of sample)}/(k_{obs} of control). A high IR value indicates strong reductive activity toward MbO_2 oxidation by the test sample. A negative IR value indicates that the sample promoted oxidation of MbO_2. The inhibitory effects of the plant extracts on the oxidation of the MbO_2 were divided into five classes: + +, strongly positive (IR=50% or more); +, weakly positive (IR=10〜49%); ±, inactive (IR=−9〜9%); −, weakly negative (IR=−49〜−10%); − −, strongly negative (IR=−50% or less).

L-Ascorbic acid contents of plants The concentration of AsA (reduced form) in the original sample solutions of Chinese cabbage, lemon, paprika, and radish were measured using the 2,4-dinitrophenyl-hydrazine method (Roe *et al.,* 1948). The absorbance was measured at 540 nm.

Results

Screening of edible plants for inhibitory effects on the

oxidation of MbO_2 Fifty-five edible plants and fungi (listed in Table 1) were investigated for reductive activities. In our previous study, we showed that the apparent rate constant value for the oxidation of MbO_2, k_{obs}, is a good index of reducing activity (Ashida *et al.,* 2004). The k_{obs} values in the presence of water extracts of edible plant samples were measured in a manner similar to that described in our previous study. The results and activities of the test plants are shown in Table 1 and Fig. 1, respectively.

Eleven (20.0%) of the 55 samples (paprika, radish, balsam pear, lemon, tomato, cabbage, Satsuma mandarin, Chinese cabbage, sweet orange, asparagus, and green pepper) were found to have strong inhibitory effects on the oxidation of MbO_2. The samples that showed strong reductive effects were a variety of fruits, vegetables, and roots. We examined the relationship between activity and sample family: most of the plants that showed high reducing activity belonged to the families cruciferae, rutaceae, and solanaceae. In contrast, 30 (54.5%) of the 55 samples promoted the oxidation of MbO_2. The majority of the plants tested promoted MbO_2 oxidation in this assay.

Measurement of the reducing effects of Chinese cabbage, lemon, paprika, and radish on the autoxidation of MbO_2 Four of the 11 strongly positive samples, representing four plant types—Chinese cabbage (leafy vegetable), lemon (fruit), paprika (fruit-vegetable) and radish (root vegetable)—were selected for more detailed investigation. The rates of inhibition of MbO_2 oxidation by water extracts of freeze-dried samples were measured at four different concentrations (1: 10, 1: 30, 1: 100, and 1: 300 dilutions). The results are shown in Table 2. The reductive effect of paprika decreased slightly as the concentration of the extract decreased from a dilution of 1: 10. The effects of Chinese cabbage and radish decreased rapidly as their concentrations were lowered, with radish becoming inactive at 1: 100 and 1: 300 dilutions. It was concluded that these samples were inferior to paprika in their reducing capabilities. Lemon exhibited a strange effect on MbO_2 oxidation: the IR value of the lemon extract was greatest

Table 1. Inhibitory effects of water extracts of edible plant samples on MbO_2 oxidation at 1: 10 dilution.

Family/species	Common name	IR(%)
Agaricaceae		
Agaricus bisporus Sing.	White mushroom	–277.6
Basellaceae		
Basella rubra L.	Indian spinach	–31.9
Chenopodiaceae		
Spinacia oleracea L.	Spinach	30.3
Compositae		
Cichorium intybus L.	Chicory	–304.5
Cichorium intybus L.(Rubifolium group)	Conical head red chicory	–433.0
Chrysanthemum coronarium L.	Garland chrysanthemum	–410.8
Lactuca sativa var capitata L.	Head lettuce (butter type)	–397.7
Lactuca sativa var capitata L.	Head lettuce (crisp type)	–398.8
Stevia rebaudiana Bertoni	Stevia	–386.3
Convolvulaceae		
Ipomoea batatas Poir.	Sweet potato	–263.7
Cruciferae		
Brassica oleracea var.italica Plen.	Broccoli	–115.8
Brassica oleracea var. capitata L.	Cabbage	57.4
Brassica pekinensis Rupr.	Chinese cabbage	56.1
Brassica campestris L. (narinosa group)	Chinese flat cabbage	–8.0
Brassica campestris var. peruviridis	*Komatsuna* (Japanese)	–77.3
Brassica chinensis L.	Qing gin cai	49.3
Raphanus sativus L.	Radish	70.3
Brassica rapa L. (Rapa Group)	Turnip	–9.1
Nasturtium Officinale R.Br.	Watercress	–9.1
Cucurbiaceae		
Momordica charantia L.	Balsam pear	62.9
Cucumis sativus L.	Cucumber	–1.9
Cucumis Melo L. *var makuwa* Makino	*Makuwauri* (Japanese)	27.4
Cucurbita maxima Duch.	Pumpkin	18.4
Cucurbita pepo var. melopepo	Summer squash	5.8
Dioscoreaceae		
Dioscorea opposita Thunb.	Chinese yam	48.9
Labiatae		
Ocimum basilicum L.	Basil	–401.4
Rosmarinus officinalis L.	Rosemary	–417.8
Salvia officinalis L.	Sage	–383.3
Mentha spicata L.	Spearmint	–394.0

at a dilution of 1: 30, in comparison with 1: 10, 1: 100, and 1: 300 dilutions. As a reduction in pH accelerates the oxidation of MbO_2, it was assumed that the addition of lemon extract caused a decrease in IR by reducing the pH of the MbO_2 solution. Indeed, it was shown that lemon extract affected the pH of the reaction solution. When the lemon extract was added at 1: 10 or 1: 30 dilution, the pH values of the reaction solutions fell to about 5.8 and 5.96 from 6.0, respectively. However, the influence on pH could be neglected at dilutions of 1: 100 and 1: 300 dilutions. It appears that acidic substances, such as citric acid in the lemon extract, which lower the pH of the test solution, will cause a decrease in reductive effect at 1: 10 dilution in comparison with the effect at 1: 30 dilution.

AsA contents in plants extracts and the influence of AsA on reducing activity AsA is a well-known reductant, and in our previous report it showed the strongest reducing capability among four compounds tested by MbO_2 assay, ferricyanide assay and XTT assay (Ashida *et al.*, 2004). AsA was considered to play a major role in the reductive effects of the edible plants on the oxidation of MbO_2 observed in the present study. To examine the contribution of AsA to the reductive activities of plants, the AsA contents in water extracts of freeze-dried sample powders (Chinese cabbage, lemon, paprika and radish) were investigated (Table 2). In this assay, paprika was found to contain more AsA than Chinese cabbage, lemon or radish. Figure 2 shows the relationship between the AsA contents in these plant extracts and their inhibitory effects. The horizontal axis represents the AsA content in the sample reaction solution, which was calculated by division of the AsA content in the original sample solution by the dilution. For comparison with the plant sample data, the IR rates of AsA as a reagent at 0.1, 0.3, 1, 3, 10, 30, and 100 μM were investigated in a similar manner, and these are also shown in Fig. 2. It is evident that the IR curves of the plant samples are similar to that of AsA, except in the case of lemon. As the IR curve of lemon was affected by the pH of the solution, as described above, the difference in that curve of lemon may be neglected. These results suggest that AsA is the main reductive substance in edible plants, as determined by MbO_2 assay. To confirm this assumption, the influences of AsOD on the IR of AsA and paprika were examined and the results

Table 1. *(continued)*

Family/species	Common name	IR(%)
Liliaceae		
Asparagus officinalis L.	Asparagus	53.4
Allium tuberosum Rottler	Chinese chives	–82.5
Allium sativum L.	Garlic (bud)	–74.8
Allium sativum L.	Garlic (bulb)	–107.8
Allium cepa L.	Onion	–244.8
Allium fistulosum L.	Scallion	–218.4
Allium fistulosum L.	Welsh onion (green type)	2.5
Rosaceae		
Malus pumila var. domestica Schneid.	Apple	–157.6
Rutaceae		
Citrus limon Burm. f.	Lemon	61.7
Citrus unshiu Marc.	Satsuma mandarin	57.0
Citrus sinensis L.	Sweet orange	53.4
Solanaceae		
Solanum melongena L.	Eggplant	–419.0
Capsicum annuum L. (*var. grossum* Sendt.)	Green pepper	53.4
Capsicum annuum L. (*var. cuneatum* Paul)	Paprika	70.8
Solanum tuberosum L.	Potato	–236.2
Lycopersicum esculentum Mill.	Tomato	60.8
Theaceae		
Thea sinensis L.	Tea	18.4
Tiliaceae		
Corchorus olitorius L.	Jew's marrow	–389.7
Tricholomataceae		
Hypsizygus marmoreus (Peck) Bigelow	*Bunashimeji* (Japanese)	20.0
Lentinus edodes Sing.	*Shiitake* (Japanese)	–256.9
Flammulina velutipes Sing.	Winter mushroom	36.0
Umbelliferae		
Angelica keiskei Koidz.	*Ashitaba* (Japanese)	–417.0
Daucus carota L.	Carrot	–181.3
Apium graveolens L.	Celery	–179.3
Cryptotaenia japonica Hassk	Japanese honewort	–299.6
Petroselinum crispum (Mill.) Nym.	Parsley	–49.9

are shown in Fig. 3. In the absence of AsA or the paprika extract, the oxidation rate of MbO_2 was not significantly influenced by AsOD. However, when AsOD was present in the reaction mixture, the IR values of the AsA and paprika solutions decreased significantly, reaching the inactive level. These observations indicated that the oxidation of AsA by AsOD resulted in the disappearance of the reductive effects of both AsA and paprika. Thus, AsA was shown to be the most important active substance in the reducing capability of edible plants such as paprika, as determined by MbO_2 assay.

Discussion

The MbO_2 assay indicated not only that various plants exhibit reducing activity, but also that some plants have pro-oxidant activity. A variety of plants, such as Chinese cabbage, lemon, paprika, and radish, were shown to have strong inhibitory effects on MbO_2 oxidation. We also found that AsA is responsible for the most of the reductive activity of plants such as paprika. However, it is notable that the majority of the plants tested promoted MbO_2 oxidation. Interestingly, plants of the compositae family such as stevia (Xi *et al.*, 1998), or the labiatae family (Nakatani, 1994), which are rich in antioxidative substances and are widely considered to be beneficial, were found to strongly promote the oxidation of MbO_2 in our assay. AsA is a common component not only of plants with reductive effects but also of those that were found to promote MbO_2 oxidation. Such pro-oxidant plants may contain strong oxidative substances that extinguish the reductive effect of AsA. It has not yet been clarified why or how some plants promote MbO_2 oxidation. We know that the oxidation of MbO_2 is promoted by a reduction in pH, as described above. However, the pH values of the

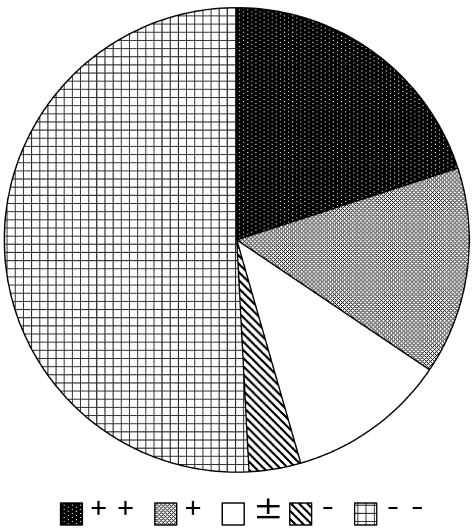

Fig. 1. Activity profile of inhibition of MbO_2 oxidation by water-extracts of edible plants (1: 10 dilution). + +, strongly active (IR=50% or more); +, weakly active (IR= 10 ～ 49%); ±, inactive (IR=−9 ～ 9%); −, weakly negative (IR=−49 ～−10%); − −,strongly negative (IR=−50% or less). Number of plants in each category: + +, 11 (20.0%); +, 8 (14.5%); ±, 6 (10.9%); −, 2 (3.6%); − −, 28 (50.9%).

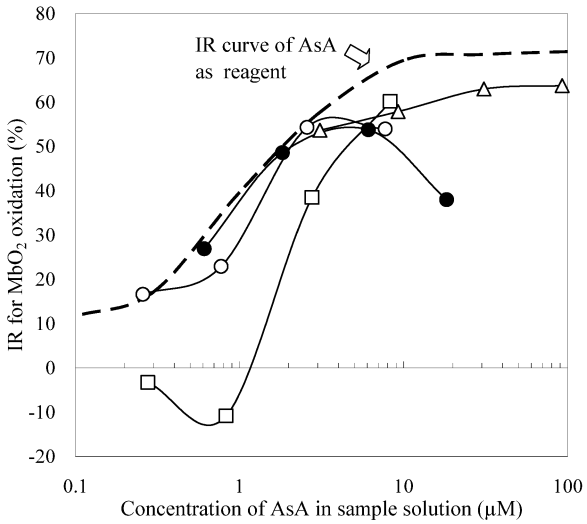

Fig. 2. Relationship between the change in IR values of reaction solutions containing Chinese cabbage (○), lemon (●), paprika (△), and radish (□), and their AsA concentrations. AsA concentrations in the reaction solutions were calculated by dividing the AsA contents in the sample original solutions (Table 2) by the dilution. The IR data are the same as those presented in Table 2. The broken line represents the IR curve of AsA as a reagent. Data represent the means of three replications.

Table 2. Comparison of inhibitory effects on MbO_2 oxidation with AsA contents of Chinese cabbage, lemon, paprika, and radish. The oxidation process of MbO_2 was carried out at 30℃ and pH 6.0. The inhibitory rate data represent the means and S.D. of three repeated experiments using different MbO_2 preparations from three different bovine muscles. The AsA concentration data represent the means of three repeated measurements.

Plant	IR on MbO_2 oxidation (%) dilution				AsA concentration in original solution(μM)
	1:10	1:30	1:100	1:300	
Chinese cabbage	53.9% ± 0.4%	54.3% ± 2.1%	22.9% ± 8.4%	16.6% ± 4.8%	77.4
Lemon	38.0% ± 4.8%	53.7% ± 3.1%	48.6% ± 4.4%	26.9% ± 11.6%	183.0
Paprika	63.7% ± 1.5%	63.0% ± 3.7%	57.8% ± 4.8%	53.6% ± 3.3%	926.0
Radish	60.2% ± 4.3%	38.5% ± 12.0%	−10.8% ± 5.6%	−3.3% ± 7.6%	82.9

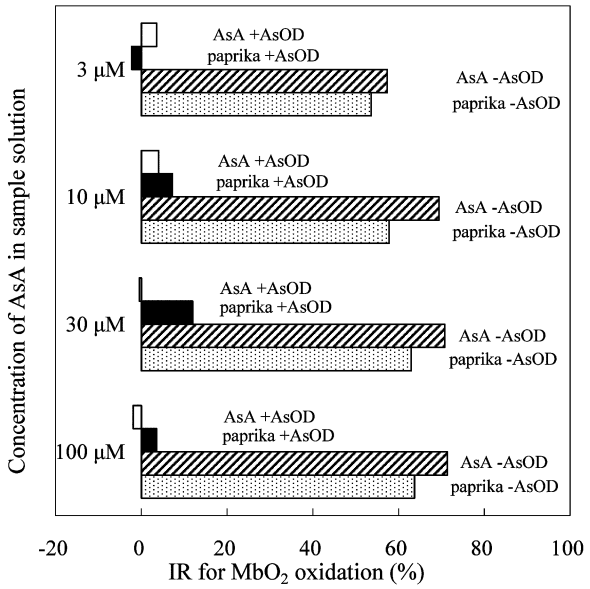

Fig. 3. Effect of AsOD on the IR values of paprika and AsA with regard to MbO_2 oxidation. The inhibition rates of the various concentrations of AsA in solutions containing paprika extract or AsA reagent are shown in the presence and absence of AsOD. The concentration of AsOD in the reaction mixture was 2.5 mg/L. The IR values for the paprika extracts and AsA without AsOD are the same data as shown in Fig. 2. Data represent the means of three replications.

reaction solutions containing extracts of pro-oxidant plants of the compositae family (stevia) and the labiatae family (basil, rosemary sage and spearmint) did not differ from that of the control solution. Metal ions such as copper (Snyder and Skrdlant, 1966), and active oxygen from such compounds as hydrogen peroxide (Yusa and Shikama,1987), also promote MbO_2 oxidation. The pro-oxidant effects of some of the plants we tested may be due to these factors. In particular, chlorophyll-related compounds may play an important role in MbO_2 oxidation, because some compounds derived from chlorophylls (such as pheophorbides) are widely known to possess the ability to generate active oxygen species such as superoxide and hydroxyl radicals.

The water extracts of various plants have been previously measured by FRAP assay, and the results were regarded as total activities of reductants (Halvorsen *et al.,* 2002). However, it is evident that plants contain numerous substances that act not only as antioxidants or reductants but also as pro-oxidants. Thus, assessment of the total reducing capacity of dietary foodstuffs by FRAP, ferricyanide, or XTT assay may result in false conclusions, because values obtained from these assays will not reflect the effects of pro-oxidants regardless of the amounts of pro-oxidants contained in the foods. The MbO_2 assay can properly assess the total reductive capacity of dietary foodstuffs because of its ability to evaluate pro-oxidant activity as well as reducing capability. To examine this assumption, we are currently planning experiments to compare the reductive capacities of foodstuffs by MbO_2 assay using data measured by other methods, such as the FRAP assay, ferricyanide assay and XTT assay. Moreover, the MbO_2 assay is widely applicable to foodstuffs other than plants.

In this study, the reductive capacities of complex systems such as crude extracts of edible plants were assessed by MbO_2 assay. These data may be useful in the identification of beneficial dietary foodstuffs for protecting against diseases in which oxidative stress is a contributing factor.

References

Arai, I. (1998). The recent level of protein-reducing substances (determined by ferricyanide reduction method) of market milk. *Shokuhin-eiseikenkyu,* **48**, 101–111 (in Japanese).

Ashida, S., Noguchi, S.F. and Sato, R.(2004). Development of a spectrophotometric assay system for evaluating reducibility of water-soluble substances. *Food Sci. Technol. Res.,* **10**, 432–436.

Benzie, I.F.F. and Strain, J.J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal. Biochem.,* **239**, 70–76.

Halvorsen, B.L., Holte, K., Myhrstad, M.C.W., Barikmo, I., Hvattum, E., Remberg, S.F., Wold, A., Haffner, K., Baugerød, H., Andersen, L.F., Moskaug, Ø., Jacobs Jr., D.R. and Blomhoff, R. (2002). A systematic screening of total antioxidants in dietary plants. *J. Nutr.,* **132**, 461–471.

Krzywicki, K. (1982). The determination of haem pigments in meat. *Meat Sci.,* **7**, 29–36.

Nakatani, N. (1994). Chemistry of antioxidants from Labiatae herbs. In "Food phytochemicals for cancer prevention II. Teas, spices and herbs." ed. by M.J. Huang, T. Osawa, C.T. Ho and R. T. Rosen, American Chemical Society, Washington DC, USA, pp. 144–153.

Roe, J.H., Mills, M.B., Oesterling, M.J. and Damron, C.M. (1948). The determination of diketo-l-gulonic acid, dehydro-l-ascorbic acid, and l-ascorbic acid in the same tissue extract by the 2,4-dinitrophenyl-hydrazine method. *J. Biol. Chem.,* **174**, 201–208.

Snyder, H.E. and Skrdlant, H.B. (1966). The influence of metallic ions on the autoxidation of oxymyoglobin. *J. Food Sci.,* **31**, 468–473.

Trout, G.R. and Gutzke, D.A. (1996). A simple, rapid preparative method for isolating and purifying oxymyoglobin. *Meat Sci.,* **43**, 1–13.

Ukeda, H., Goto, Y., Sawamura, M., Kusunose, H, Kamikado, H. and Kamei, T. (1995). Reduction of tetrazolium XTT with UHT-treated milk: Its relationship with the extent of heat-treatment and storage conditions. *Food Sci. Technol. Int.,* **1**, 52–57.

Xi, Y., Yamaguchi, T., Sato, M. and Takeuchi, M. (1998). Antioxidant activity of *Stevia rebaudiana*. *Nippon Shokuhin Kagaku Kogaku Kaishi,* **45**, 310–316 (in Japanese).

Yusa, K. and Shikama, K. (1987). Oxidation of oxymyoglobin to metmyoglobin with hydrogen peroxide: involvement of ferryl intermediate. *Biochemistry,* **26**, 6684–6688.